HIRUDINEEN STUDIEN.

PRELIMINARY NOTICE.

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THE complete paper which will be published under the above title was finished in April, 1896. Many difficulties have arisen with regard to its appearance in print, and when it will be given to the public is as yet entirely uncertain.

The fact that certain of the problems which I tried to solve are being worked out by at least one other author compels me to communicate the more important of my results in a condensed preliminary form.

The primary object of my paper was to investigate the anatomy of the excretory organs of Nephelis and Clepsine. During the progress of the work, however, it became evident that without an insight into the finest structure of the nephridial cells no inferences could be drawn as to the physiology of excretion.

On the other hand, it was seen that without a fair knowledge of the general organization of these animals no deeper anatomical understanding of the organs in question could be gained, and this conviction induced me to devote some time to the study of the other organs. During this study it was found that the lymphatic cells stand in intimate relation to excretion, and considerable time was given to the investigation of these cells.

The whole paper was finally divided into:

1. A short account of the general organization of Nephelis and Clepsine.
2. The anatomy of the excretory organs.
3. The cytology of the excretory organs.
4. The physiology of excretion.
5. The origin of the pigment.
6. The cause of the color pattern in the skin.

The subjects of the last two items have been shortly dealt with in a preliminary account which appeared in the *Zool. Anz.*, No. 468, February, 1895, "Ueber den Ursprung des Pigments und der Zeichnung bei den Hirudineen."

The physiology of excretion has been made the subject of a lecture at the Marine Biological Laboratory at Wood's Holl, Mass., in August, 1896, which will be published in the volume of lectures for 1896 and 1897.

The few new points in No. 1 I shall not record here, and it therefore only remains to me to outline my results on the anatomy and cytology of the nephridium.

I. *Anatomy of the Nephridium of Nephelis and Clepsine.*

The following terms have been proposed for the different parts of the nephridium of these forms:

1. Portio afferens: the funnel apparatus.

2. Portio afferens-glandulosa: the part of the nephridial gland which both receives excretory products and which at the same time by its own chemical activity produces oxydized end-products.

3. Portio glandulosa-efferens: the part of the nephridium which has, besides the chemical excretory function, the task of conveying excretory end-products to the outside.

4. Portio efferens: the terminal vesicle and the terminal canal, opening at the surface of the animal to the exterior.

The portio afferens. — The funnel apparatus consists of two distinct parts: the crown and a vesicle.

The funnel crown is formed in Nephelis by from five to eight bilobed ciliated cells grouped around a central lumen. The cells are all strongly curved to the outside and downwards, and the funnel crown may in its shape be best compared with the flower of a tiger-lily (Lilium tigrinum or Lilium martagon). The crown cells are covered with cilia on the convex (upper and inner) surface, and these cilia produce by their motion a stream running centripetally, by which little granules floating in the surrounding liquid are conveyed into the central opening.

In Clepsine the funnel consists of three cells, two crown cells and one peduncle cell. The crown cells are differently shaped in different species, but they all more or less resemble the crown cells of Nephelis. The peduncle cell is one long cylindrical cell with an intracellular lumen, which lumen is lined with long cilia.

The crown cells are situated opposite each other on the outer end of the peduncle cell, and the whole funnel crown therefore resembles the letter T.

I have been fortunate enough to determine the mode of growth of the funnel cells by dissecting out funnels of very young and of very old specimens.

The vesicle, which is the second important part of the funnel apparatus, has the same appearance in all the leeches. I propose for it the term "receptaculum excretorium." It is a hollow vesicle, the walls of which are composed of flattened connective-tissue cells, and it is only open toward the central opening of the funnel crown, and closed *in all other places.*

The connection of this vesicle with the funnel crown cells has been generally misunderstood.

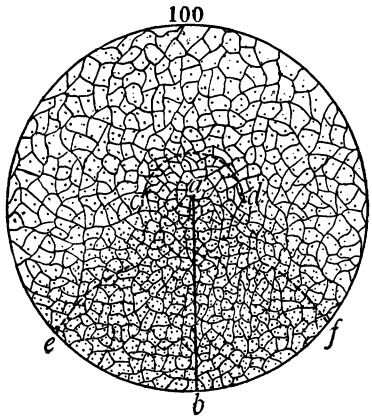
The connective-tissue wall of the receptaculum is continuous with a connective-tissue membrane covering the entire outer and under surface of the crown cells in Nephelis and enveloping the whole peduncle cell in Clepsine. Thus the ciliated funnel crown is entirely wedged in with its unciliated surface into the receptaculum, as the petals of some flowers are imbedded in the calyx leaves.

In very young funnels of Clepsine I have found that both the peduncle cell and the receptaculum are extremely minute, the receptaculum being hardly demonstrable, and thus the conclusion was easily drawn that this receptaculum is only a by-product in the process of growth of the funnel proper, growing with the increase of excretion (see Physiology of Excretion). That the receptaculum is a hollow vesicle filled with débris was proved by pricking it with a needle, when the contents were seen flowing out, while the walls collapsed.

The funnel of Nephelis lies in the first ampulla of the segment, whereas in Clepsine the position of the funnels is very

variable. In some species it lies in the ventral lacuna near the ganglionic chain; in others it is situated in a lateral pouch of this ventral lacuna; in others it still lies ventrally in the so-called intermediate lacuna (Zwischen lacune) of Oka; and, finally, it may assume a dorsal position in a coelomic section near the lateral lacuna.

In Clepsine Hollensis the funnels of the anterior segments lie near the ventral lacuna, whereas in the posterior segments



they lie dorsally, very near the lateral lacuna; a fact which would imply that the more primitive position is the ventral one.

The other part of the nephridium of both genera consists of a chain of cells possessing a different structure in different parts.

The portio afferens-glandulosa, the innermost cells of which stand in close contact with the receptaculum (not in open communication, however), is characterized by an intricate network of vacuoles and canals filling the cells of that portion. The portio glandulosa-efferens is pierced by a single central canal only. Both portions are covered with a connective-tissue membrane.

The whole course of the nephridium with all its intricate windings and loops was outlined with the help of the camera lucida from a very young specimen of Clepsine Hollensis lightly

pressed under a cover glass while living, and it was found that the shape of the nephridium adapts itself to the shape of the spaces left free by the surrounding tissues. It is impossible and idle to establish morphological types of nephridia for the different genera of leeches.

The vesicula terminalis is lined by an epithelium-bearing cilia on the inner surface. The canalis terminalis is to be regarded as a simple invagination of the epidermis.

The anatomical investigation was almost exclusively prosecuted on living material, partly on very young uninjured animals, partly by the study of rapidly made dissections and teased preparations. This was of paramount importance in order to gain perfectly trustworthy results and to eliminate the subjective interpretation of reconstructions from sections. This anatomical investigation was finished in all its details in the fall of 1894, and all the drawings pertaining to this part of the work were executed in Wood's Holl during the summer of 1894.

In the fall and winter of 1894 the investigations concerning the pigment formation and origin of markings were finished, and in the spring and summer of 1895 the greatest part of the cytology of the nephridium was worked out.

II. *The Cytology of the Excretory Organs.*

New points were gained with regard to the finest structure of the ciliated cell by the study of the ciliated crown cells of the funnel.

The cilia were found to consist of three parts, a basal piece staining deeply with haematoxylin, a middle piece staining very faintly with the same agent, and the flagellum or cilium proper staining with the acid anilins, *e.g.*, Bordeaux red. The basal piece is rod-shaped, and stands not quite vertically to the ciliated surface, but a little inclined. The middle piece is more round, and it seems as if the middle pieces of one row of cilia touched each other. The flagellum is thin, very long, and elastic, and is regarded by the author as a passive material, a metamorphosed protoplasm, like a secretion.

The cause of the ciliary motion is to be sought in the contraction of the basal piece, the middle piece playing a nervous rôle.[1]

This structure seems to be of general occurrence, as the writer found it also, during the last winter, in the ciliated intestine cells of the leeches in all its details.

The cytoplasm of the ciliated funnel cell is specifically modified, inasmuch as the cytoplasmic threads are arranged parallel to each other, each cilium standing in connection with one of these threads.

It was observed at first that near the edge of the ciliated cell, where the basal pieces of the cilia stand in communication with the cytoplasmic threads, a great number of little granules are assembled which make this connection obscure, and it was at first inferred that those granules all consisted of food matter destined for the regeneration of the basal pieces. I have, however, after the study of the cells of the intestine in these animals, altered my views in that respect. Only a portion of these granules is food material. The basal piece of the cilium is connected with a fine, short thread projecting into the cell and ending inwardly in a thick granule. (These granules have been discovered and described by me as peripheral organs in other cells.) Only this granule is connected with the cytoplasmic thread.

The function of these peripheral organs is a problem of very great interest, but I must leave a discussion of this to a later time, when I have completed my observations.

The cytoplasmic threads of the ciliated cell are all parallel and show not the slightest connection in their arrangement with the nucleus.

The nucleus is large, shows a very clear, distinct linin network, and the chromatin is suspended in that network in the form of little granules.

The cells of the portio afferens-glandulosa. — The row of cells forming that part of the nephridium which I call portio afferens-glandulosa presents under the microscope very various structures, according to the place from which they are taken.

[1] See Physiology of Excretion.

The cells nearest to the receptaculum are very large, possess a large nucleus with loose linin network, very large nucleoli, and loosely scattered chromatin granules. The membrane of the nucleus is very thin. The shape of the latter is very irregular, numerous processes projecting all over the surface.

The cytoplasm appears as a beautiful clear network with rather wide meshes. This network is not, as Bolsius states, radially arranged, but it is equally well developed in every direction. The microsomes cluster around the cytoplasmic threads.

These cells are filled with two kinds of vacuoles, some large ones near the periphery and great masses of exceedingly small ones near the center of the cell.

In the cells farther away from the receptaculum the peripheral vacuoles have partly fused together, forming an irregular network of canals, and the vacuoles of the central masses also fuse more and more together and ultimately form one single central canal in the cell.

As soon as this central canal is definitively formed a new group of structural elements becomes conspicuous in the cell, namely, the peripheral organs.

They appear at first only in the shape of microsomes, a trifle larger than the other microsomes of the cell and more regularly arranged along the inner edge of the cell surface. The farther away we go from the receptaculum the larger these peripheral microsomes grow and the more regularly they are arranged, until they become finally eight to ten times as large as the other microsomes and are each of them attached by means of a thick, strongly staining cytoplasmic thread to the surface of the cell. In the meantime the central canal becomes a wide tube (in some cases bifurcating in places), and the network of peripheral canals becomes united with the central canal, forming elegantly ramifying side branches of the latter.

The cells of the portio glandulosa-efferens next to these are pierced only by the central canal, which shows no more bifurcations and possesses no more side branches.

The cells are smaller than those of the portio afferens-glandulosa, as are also the nuclei, which are perfectly smooth and

without processes. The nuclear membrane is thicker, the chromatin granules more crowded.

The central canal is surrounded by a cuticular layer in which the intracellular musculature, consisting of parallel rings studded with granules and united by cross anastomoses, is developed.[1]

The peripheral organs are best developed in the cells of this part of the nephridium.

There is nothing new to be said with regard to the structure in the terminal vesicle and the terminal canal.

The main point of the cytological part of the paper is the demonstration of true organs in the cell which develop gradually under a gradually increasing stimulus.[2]

With regard to the rest of the paper, I simply mention that it chiefly deals with the distribution of the pigment and with the cytology and physiology of the elements connected with that process.

[1] See Physiology of Excretion. [2] *Ibid.*